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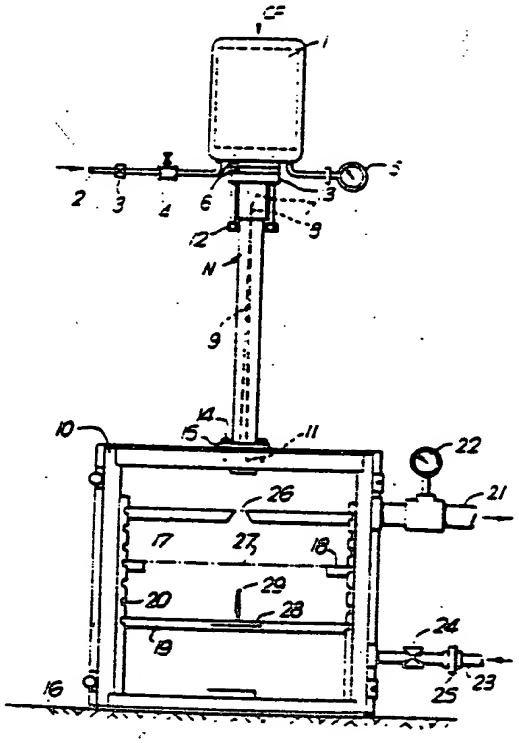
(57) Abstract

Apparatus for, particularly, penetrating living cells with genetic material by bombarding the cells with DNA coated microprojectiles comprises a gas pressurizable reservoir (1) connected to a nozzle (N) which leads to a target chamber (10) containing a target station (28). A membrane seals the upper end of the nozzle from the reservoir and microparticles are supported on the upstream side of the membrane. Upon increase in the gas pressure in the reservoir, the membrane ruptures and the particles are projected at supersonic speeds through the nozzle (N), into the chamber (10), and bombard a target at the station (28). Target disturbance is minimised by a flow expander plate (17) and an obstruction (29).



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BALLISTIC APPARATUS

This invention relates to apparatus for propelling microparticles at high velocity towards a target. More specifically the invention relates to improvements in the high velocity delivery of particles to a target of living cellular material for the purpose of penetrating the cells with the particles in order to transform the cells with an exogenous gene sequence introduced via an aperture created by the particles on impact.

A method is known whereby living cells may be genetically transformed by delivering a particle, typically of a dense material such as tungsten or gold, into the cell in culture. A foreign gene may be loosely adhered to the particle so that it is carried into the cell as the particle penetrates. Alternatively, the foreign gene may be added to the culture which is then bombarded with untreated particles. The foreign gene then enters the cell by diffusion through the aperture created on impact of the particle.

In such microballistic methods, the ability of the particles to penetrate the cell membrane or cell walls is dependent upon the velocity and density of the particles. The density of the materials commonly used, tungsten and gold, cannot easily be increased as these represent the densest available materials which are readily available in the very fine particle size which is necessary, around 1 to 2 μm. The usually supersonic velocity at which the particles are propelled is, in practical terms, the limiting factor. The two known methods employ either an explosive charge or an explosively volatilised liquid droplet to provide the propulsive force. Both of these do have disadvantages, one of which is poor distribution of the particles over the target cell culture area and frequent destruction of the culture in a generally central region of the impact. Blast effects also tend to disperse the culture.

According to a first aspect of the present invention, an apparatus for the high velocity propulsion of microparticles comprises an elongate tubular device, a pressurizable gas reservoir connected to one end of the device, means between the device ends, for holding particles to be propelled, a target station adjacent to the other end of the device and a membrane located between the device ends, the membrane being rupturable on application of a predetermined pressure of gas from the reservoir.

Preferably the tubular device includes a convergent/divergent nozzle interposed between the membrane and the target station. The design of the nozzle is intended to accelerate expanding gas and the divergent taper in the nozzle improves the flow characteristics to laminar conditions.

The means for holding the microparticles, which may be metal particles or water droplets possibly frozen during projection or flight to increase target penetration, could involve immobilising them, e.g. electrostatically on or upstream of a rupturable membrane, which is ruptured when the gas flow commences, to release the particles into the gas stream. The rupturable diaphragm may be the same as the rupturable diaphragm which ruptures to initiate the gas flow from the reservoir chamber. Alternatively the particles could be injected into the gas stream through a hollow needle. This technique would be particularly appropriate for introducing an aerosol spray of an aqueous/DNA solution.

A section of the tubular device downstream of the membrane and containing the target station is preferably evacuable. The apparatus may include a target holder adjustable within the tubular device to decrease or increase the distance between the membrane and the target.

A potential problem with this technique for bombarding e.g. living cells is that loosely adherent cells or other targets tend to be dislodged from the target station by the

gas flow which is deflected laterally by and over the target.

According to a second aspect of the present invention, apparatus for high velocity propulsion of microparticles comprises a target station, a nozzle for directing a supersonic gas flow towards the target station, a source of high pressure gas coupled to the nozzle, means for introducing microparticles so that they are carried in the gas flow towards the target station, and an obstruction positioned in front of the target station for intercepting the approaching gas flow and producing a shock wave which deflects the gas flow substantially past the target station, whilst substantially not interfering with the trajectories of the microparticles towards the target station. This deflection of the gas flow greatly reduces cell or other target blow away from the target station.

This aspect is useful not only when the supersonic gas flow is produced by a rupturable membrane. Alternative high pressure gas sources could be an explosive charge or a reservoir which is pumped up and from which the gas is suddenly released by the sudden opening of a valve.

The obstruction may be a spike which projects axially from the target station towards the nozzle, and which causes a shock wave to form. This conical shock wave deflects the gas flow radially outwards and provides a conical dead-gas zone ahead of the target station, so that target disturbance is minimised. In round figures, release of air at a pressure of 5 bar in a reservoir chamber, through a suitable convergent-divergent nozzle into a target chamber held at a vacuum of 0.5 bar, can produce air velocities of twice the speed of sound (mach number of 2), and the conical shock wave generated by this spike provides a conical dead-air zone of semi-angle about 17°, which is sufficient to deflect the airflow past at least that part of a typical target on which e.g. the cells are immobilised. The cells or other target is/are thus substantially not disturbed by the airflow whereas, e.g.

tungsten, particles, having a density four orders of magnitude greater than that of air continue to fly with their trajectories not significantly changed, until they impact the target.

As an alternative to a spike, the obstruction could take the form of a bar having a width smaller in comparison with the working area of the target, extending transversely across the centre of the gas flow, so that the airflow would be deflected as a wedge, rather than as a cone. The bar could be supported at its ends on the opposite sides of the chamber in which the target is positioned.

Target disturbance can be further reduced by the provision, between the nozzle and the target station obstruction, of a flow expander in the form of a diverg nt, e.g. frusto-conical, orifice in a plate, which is preferably positioned in a target chamber.

An example of an apparatus constructed in accordance with the present invention is illustrated in the accompanying drawings, in which:

Figure 1 is a diagrammatic central vertical secti n through the apparatus; and,

Figure 2 is an enlargement of part of Figure 1.

The illustrated apparatus has a reservoir 1 for air under pressure. The reservoir can be pressurized, through a line 2 containing an on/off needle valve 3 and a relief valve 4, to a pressure displayed by a gauge 5. An outlet at the bottom of the reservoir is sealed to the upper end of a tubular device made up of a stack of a module of two annular plates 6, a nozzle N having a convergent porti n 7 leading through a throat 8 to a divergent portion 9, and a target chamber 10 to an inlet 11 of which the lower end of the nozzle is sealed.

The stack is aligned at the top by pins 12 passing through holes in a flange 13 at the top of the nozzle N, and in th plates 8 and into blind bores in the bottom wall of the reservoir around the utlet. Similarly the stack is aligned at the bottom by pins 14 passing thr ugh holes in

a flange 15 at the bottom of the nozzle into blind bores in the top wall of the chamber 10. The parts are sealed by O-rings interposed between the reservoir 1, plates 5 and flange 13, and between the flange 15 and chamber 10. The stack is cramped tight by a downward force, shown diagrammatically by the arrow CF, working against the reaction of a supporting surgace 16 for the chamber 10.

The chamber 10 is generally cubic in shape and has a hinged perspex door for the insertion and removal at selected heights in the chamber below the inlet 11 of a flow expander plate 17, a mesh-supporting frame 18 and a target station plate 19, the edges of which are slid into selected grooves in racks 20 mounted in the sidewalls of the chamber. The chamber can be evacuated through a line 21 to a pressure displayed by a gauge 22, and can be refilled with air through a line 23 under the control of a valve 24, the line containing a filter 25. The chamber, after the door has been closed and sealed, can be evacuated to 0.9 bar vacuum in under one minute. The filter 25 is a 0.2 μm airfilter to prevent microbes from entering the sterile system.

The flow expander plate 17 has a frusto-conical flow expander orifice 26 of substantially 20° semi-angle, an inlet diameter of 10 mm, an exit diameter of 19 mm, and a length of substantially 10 mm.

The frame 18 supports the edge of a fine mesh screen 27.

The target station plate 19 carries at its centre a target station 28 fitted centrally with an upstanding spike 29 which is shown in more detail in figure 2.

When the apparatus is prepared for use, a rupturable Mylar membrane is fitted between, and forms a preassembled module with, the tw clamping plates 6, so as to ext nd across and seal the outlet from the reservoir 1 to the nozzle N. Particles, such as tungsten particles are temporarily immobilised on the upstream face of the membrane and the module is inserted laterally between the

reservoir and the flange 13 whereupon the pins 12 are inserted and the stack is cramped up. A target t be bombarded is placed on the support 28, and the chamber 10 is then sealed and evacuated to appropriate vacuum by a vacuum pump acting through the line 21. The pressur in the reservoir 1 is then raised, by opening the valve 3, and when the pressure in the reservoir has reached a threshold value, it ruptures the membrane and accelerates the tungsten particles to supersonic speeds through the nozzle N and into the chamber 10. The jet of entrained particles is aimed at the aperture 26. The flow of air diverges mor than the stream of heavier particles and air flow passing through the aperture is deflected by up to 20° by expansion waves at the aperture inlet. Flow impacting the top surface of the flow expander plate is deflected radially outwards, through shock waves. The effect of this is further to accelerate the microparticles from, for example, a Mach number of 2.2 at the chamber inlet 11 to a Mach number of about 3.1 after passing through the aperture 26. These accelerated microparticles then bombard the target whilst the capacity of the airflow to dislodge the target has been reduced. The chamber 10 is opened after closing the valve 3 and opening the valve 24, and the target is recovered, whereafter the apparatus is set for the next cycle of operation.

The spike 29 also contributes to the reduction in the disturbance of the target by the supersonic airflow in which the particles 30 are entrained. Thus, as shown diagrammatically in Figure 2, the supersonic airflow 31 impinges on the tip of the spike and the conical shock wave 32 generated by this spike provides a conical dead-air zone 33 of semi-angle of about 17°, which is sufficient to lead deflected air 34 past at least the central portion of a target on the station 28 whilst the tungsten particles 30 continue towards the centre of the target substantially undeflected.

The purpose of the mesh screen 27 is to break up any large conglomerates of tungsten particles.

The illustrated apparatus is suitable for use in gen expression, in which case, for example, the tungsten microparticles might be coated with appropriate DNA, with a target consisting of maize cells immobilised by an alginate adhesive on a filter paper resting on the target station 28. The DNA coating may provide sufficient tackiness to cause the particles to adhere lightly to the membrane prior to rupture.

CLAIMS

1. Apparatus for the high velocity propulsion of micr - particles, the apparatus comprising an elongate tubular device (6,N,10), a pressurizable gas reservoir (1) connected to one end of the device, means (6) between the device ends for holding particles to be propelled, a target station (28) adjacent to the other end of the device, and a membrane located between the device ends, the membrane being rupturable on application of a predetermined pr ssure of gas from the pressure reservoir.

2. Apparatus according to claim 1, in which the means for holding the microparticles comprises a rupturable membrane which is arranged to be ruptured when the gas flow commences.

3. Apparatus according to claim 1 or claim 2, wherein the tubular device includes a convergent/divergent nozzle (N) interposed between the membrane and the target station (28).

4. Apparatus according to claim 3, in which an obstruction (29) is positioned in front of the target station (28) for intercepting the approaching gas fl w and producing a shock wave which deflects the gas fl w substantially past the target station, whilst substantially not interfering with the trajectories of the microparticl s (30) towards the target station.

5. Apparatus according to claim 4, in which the obstruction is a spike (29) which projects axially from the target station (28) towards the nozzle (N).

6. Apparatus according to claim 4 or claim 5, further comprising a flow expander in the form of a plate (17) with

a divergent orifice (26) interposed between the nozzle (N) and the obstruction (29).

7. Apparatus according to any one of claims 4 to 6, wherein the target station (28), the obstruction (29), and, when provided, the flow expander plate (17), are located in a vacuum chamber (10) to which the nozzle is sealed.

8. Apparatus for the high velocity propulsion of microparticles, the apparatus comprising a target station (28), a nozzle (N) for directing a supersonic gas fl w towards the target station, a source (1) of high pressure gas coupled to the nozzle, means (8) for introducing microparticles so that they are carried in the gas flow towards the target station, and an obstruction (29) positioned in front of the target station for intercepting the approaching gas flow and producing a shock wave which deflects the gas flow substantially past the target station, whilst substantially not interfering with the trajectories of the microparticles (30).

9. Apparatus according to claim 8, in which the obstruction is a spike (29) which projects axially from the target station (28) towards the nozzle (N).

10. Apparatus according to claim 8 or claim 9, further comprising a flow expander in the form of a plate (17) with a divergent orifice (26) interposed between the nozzle (N) and the obstruction (29).

11. Apparatus according to any one of claims 8 to 10, wherein the target station (28), the obstruction (29), and, when provided, the flow expander plate (17), are locat d in a vacuum chamber (10) to which the nozzle is sealed.

Fig. 1.

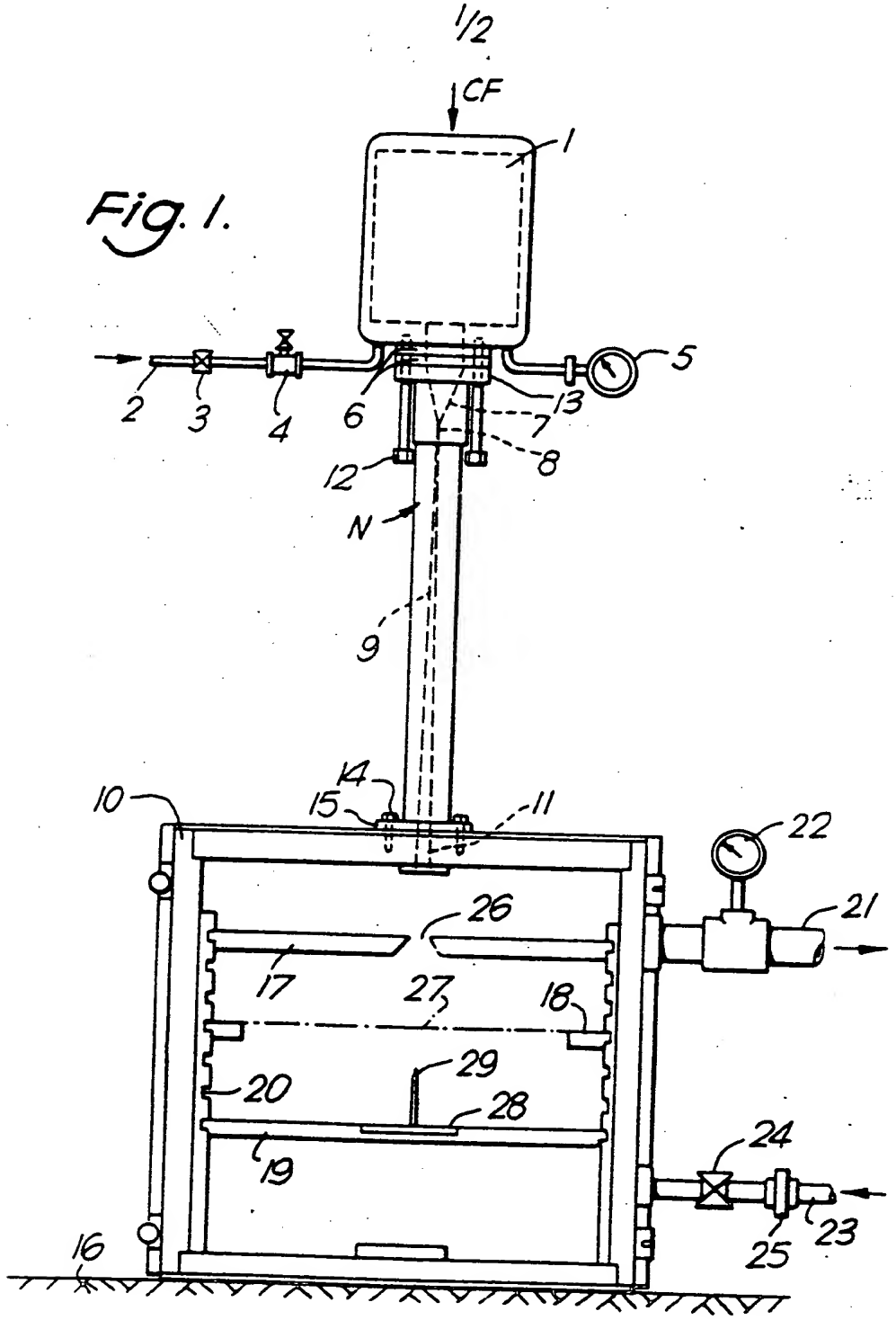
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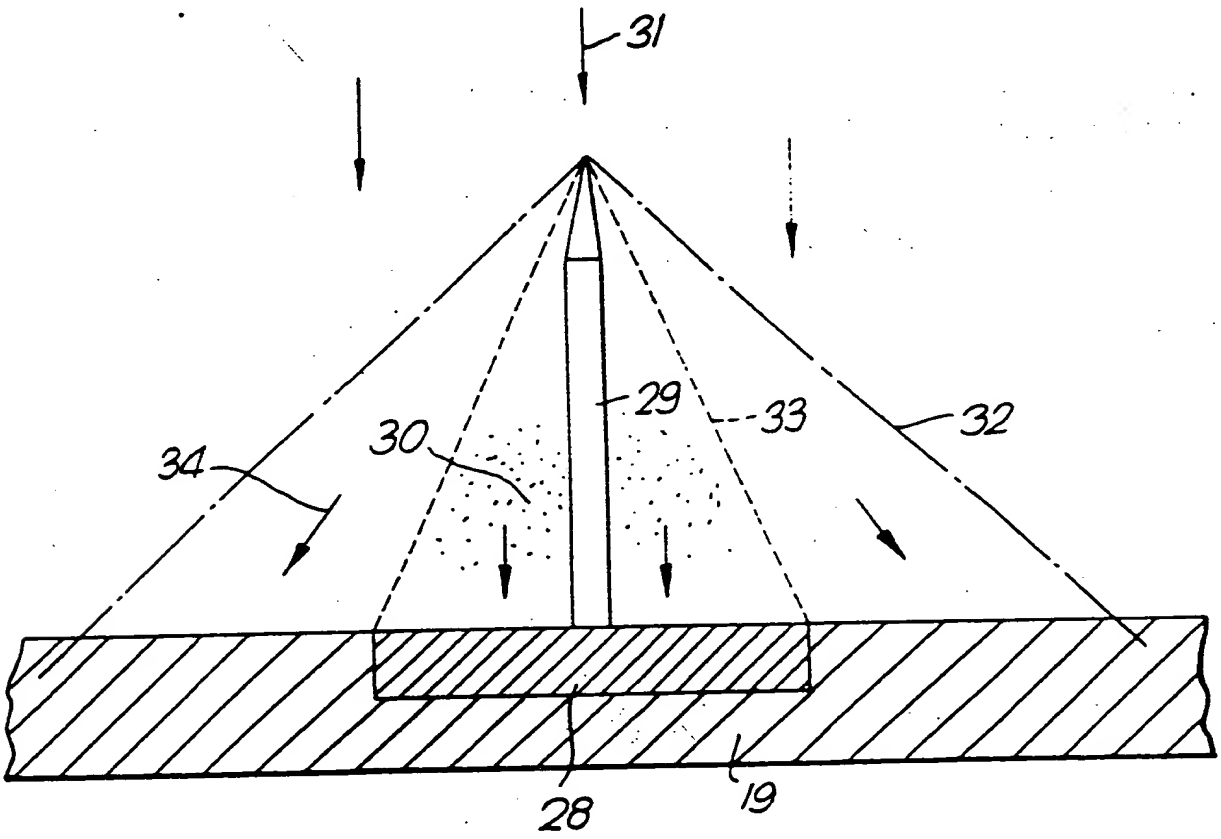
Fig. 2.



INTERNATIONAL SEARCH REPORT

PCT/GB 91/01470

International Applicants

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12M3/00; C12N15/87

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12M ; C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	PLANT PHYSIOLOGY vol. 92, 1990, BETHESDA, MD pages 334 - 339; J.H. OARD ET AL.: 'Transient Gene Expression in Maize, Rice and Wheat Cells Using an Airgun Apparatus.' see page 335, column 2, paragraph 2 - page 336, column 2, paragraph 1 see page 337, column 1, paragraph 2 - column 2, paragraph 2 --- -/--	1,2,7

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10 DECEMBER 1991	23.12.91
International Searching Authority	**Signature of Authorized Officer**
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III. DOCUMENTS CONSIDERED T BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, no. 7, April 1991, WASHINGTON US pages 2726 - 2730; R.SANDERS WILLIAMS ET AL.: 'Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles.' see page 2726, column 2, paragraph 2 - page 2727, column 1, paragraph 2; figure 1	1-4,6-8, 10-11
P,Y	WO,A,9 100 915 (BIOTECHNOLOGY RESEARCH & DEVELOPMENT CORPORATION) 24 January 1991 see claims 1-13,49-54	1-4,6-8, 10-11
A	PARTICULATE SCIENCE AND TECHNOLOGY vol. 5, no. 1, 1987, HEMISPHERE PUBLISHING CORPORATION pages 27 - 37; J.C. SANFORD ET AL.: 'Delivery of substances into cells and tissues using a particle bombardment process.'	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101470 SA 51053

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 10/12/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9100915	24-01-91	AU-A- 5950090	06-02-91